Short communication

Hydrogen sulfide producing enzymes in pregnancy and preeclampsia

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ABSTRACT

Preeclampsia, a human pregnancy specific disorder is characterized by an anti-angiogenic state. As hydrogen sulfide (H2S) has pro-angiogenic and anti-oxidative characteristics, we hypothesized that H2S levels could play a role in the pathogenesis of preeclampsia and studied the placental expression of the H2S-producing enzymes cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS). CBS and CSE protein are expressed in the fetal-placental endothelium and CBS only in Hofbauer cells. CBS mRNA expression is decreased (p = 0.002) in early-onset preeclampsia, while CSE mRNA is unchanged. Thus, down regulation of CBS during early-onset preeclampsia may result in less H2S-production and may aid in the anti-angiogenic state.

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1. Introduction

Preeclampsia (PE), a human pregnancy specific disorder, is characterized by placental ischemia and maternal endothelial dysfunction [1]. The poorly perfused and ischemic placenta releases excess amounts of anti-angiogenic factors causing generalized endothelial damage [2,3]. Hydrogen sulfide (H2S) is produced from the amino acid L-cysteine by two pyridoxal 5’-phosphate-dependent enzymes cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS). H2S induces vasorelaxation by opening ATP-sensitive K-channels in smooth muscle cells and up regulates vascular endothelial growth factor [4,5]. Furthermore, H2S also has antioxidant capacity by direct scavenging of nitrogen or reactive oxygen species [6]. CBS and CSE are down regulated in several cardiovascular and pulmonary diseases [7]. Exogenous H2S (NaHS) administration is proposed as a novel therapy in animal models of cardiovascular and ischemic diseases [7,8]. CBS is also an important enzyme in the homocysteine pathway, since homocysteine is converted to cystathionine by CBS. Pregnant CBS transgenic mice show a moderate increase of homocysteine which associated with blunted endothelial-dependent relaxation in arteries [9].

We hypothesized that H2S, because of its pro-angiogenic and anti-oxidative characteristics and the involvement of CBS in homocysteine degradation, might play a role in the pathogenesis of PE. The aim of the present study was to identify and compare the expression and localization of CBS and CSE in placental tissue from both normotensive and early- and late-onset PE.

2. Methods

Placental biopsies were obtained from patients (n = 36) with early-onset PE, late-onset PE and mode of delivery matched healthy pregnant controls after informed consent. The local UMCG Medical Ethical Committee approved the study. PE was defined according to the standards of the International Society for the Study of Hypertension in Pregnancy: diastolic blood pressure of >90 mmHg and proteinuria >300 mg/24 h [10]. PE present before 34 weeks of gestation was defined as early-onset, these patients delivered by Cesarean section. Intra-uterine growth restriction (IUGR) was defined as birth weight under the tenth percentile (Table 1).

As previously described, placental cryosections were stained [11] with mouse monoclonal antibodies against CSE (1:100, donated by Dr. N. Nishi, Kagawa Medical School, Japan) and CBS (1:250, Abnova, Taipei, Taiwan). Primary antibody was replaced by PBS in negative controls. For immunofluorescence double staining, CD31 (1:100, Sigma–Aldrich, St. Louis, MO) was used.

For real time RT-PCR, RNA was isolated from several parts of the placenta, pooled, and purified as previously described [12]. We analyzed mRNA expression of CBS and CSE using Assay-on-Demand Gene Expression (Applied Biosystems, USA).
et al. showed that H2S is endogenously produced in the placenta, nous H2S in PE needs to be elucidated.

Activity and H2S-production, while protein levels did not [16].

Brain tissue, decrease in mRNA corresponded to decreased CBS-

demonstrated that CBS and CSE are mainly localized in the endo-

thelium in the fetal vessels from the chorionic- and stem-villi

from pregnancies complicated by early-onset PE when comparing

gene, the expression of this gene was constant over the four study groups[13].

PSMD4 (proteasome non-ATPase regulatory subunit 4) was used as a housekeeping
gene, expression of this gene was constant over the four study groups.[13].

Total proteins from placental biopsies were extracted and western analysis was

performed according to published procedures [14,15]. CBS and CSE monoclonal

antibodies (Abnova) were used at 1:500 dilution.

For statistical analysis Mann–Whitney U test was used. Quantification of protein

expression was performed by measuring band intensity using ImageJ software.

Table 1

Clinical characteristics of pregnant women with early- and late-onset preeclampsia and mode of delivery matched controls.

<table>
<thead>
<tr>
<th></th>
<th>Control delivery by Cesarean section</th>
<th>Early-onset preeclampsia</th>
<th>Spontaneous delivery</th>
<th>Late-onset preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control pregnancy</td>
<td>Early-onset preeclampsia</td>
<td>Spontaneous delivery</td>
<td>Late-onset preeclampsia</td>
</tr>
<tr>
<td>Number</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33 (26–35)</td>
<td>31 (25–26)</td>
<td>27 (27–37)</td>
<td>30 (26–33)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks + days)</td>
<td>38 + 6 (38 + 6–39 + 3)</td>
<td>28 + 6 (27 + 5–30 + 3)**</td>
<td>39 + 3 (38 + 5–41 + 0)</td>
<td>38 + 1 (37 + 0–39 + 3)**</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132 (125–147)</td>
<td>178 (166–190)*</td>
<td>120 (118–120)</td>
<td>155 (150–163)**</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 (80–85)</td>
<td>110 (110–115)**</td>
<td>76 (70–80)</td>
<td>97 (92–105)**</td>
</tr>
<tr>
<td>Proteinuria (grams/24 h)</td>
<td>0</td>
<td>3.0 (0.9–4.9)**</td>
<td>0</td>
<td>0.8 (0.7–0.8)**</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3880 (3610–4085)</td>
<td>933 (713–1103)**</td>
<td>3390 (3180–3700)</td>
<td>2770 (2569–3071)**</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IUGR</td>
<td>0</td>
<td>2 (20)</td>
<td>0</td>
<td>1 (11)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) and numbers (%). For statistical analysis Mann–Whitney U test and Fisher exact test were used.

*p < 0.05, **p < 0.001, when compared to healthy pregnancy with delivery by Cesarean section.

*p < 0.05, **p < 0.001, when compared to healthy pregnancy with spontaneously delivery.

PSMD4 (proteasome non-ATPase regulatory subunit 4) was used as a housekeeping
gene, expression of this gene was constant over the four study groups [13].

Total proteins from placental biopsies were extracted and western analysis was

performed according to published procedures [14,15]. CBS and CSE monoclonal

antibodies (Abnova) were used at 1:500 dilution.

For statistical analysis Mann–Whitney U test was used. Quantification of protein

expression was performed by measuring band intensity using ImageJ software.

3. Results and discussion

The major finding of this study is that CBS mRNA expression is

significantly down regulated in placental villous tissue derived

from pregnancies complicated by early-onset PE when comparing

to mode of delivery matched controls (Fig. 1A). Furthermore, we demonstrated that CBS and CSE are mainly localized in the endo-

thelium in the fetal vessels from the chorionic- and stem-villi

(Fig. 2A). The endothelial origin of both enzymes is confirmed by
double staining with CD31 (Fig. 2B). Hofbauer cells express CBS

(Fig. 2A). There were no differences in CBS/CSE in protein expres-
sion between PE and delivery matched controls (Fig. 1B). However,

CBS/CSE protein expression was significantly down regulated in all

placentae after spontaneous delivery compared to Cesarean
delivery (Fig. 1B).

Although protein expression of CBS and CSE was not affected by

PE, we found a down regulation of mRNA of CBS in early-onset PE.

This discrepancy between mRNA and protein expression is

remarkable, but has been reported previously [16]. In ischemic

brain tissue, decrease in mRNA corresponded to decreased CBS-

activity and H2S-production, while protein levels did not [16].

Since protein levels do not imply protein-activities, we did not evaluate CSE- and CBS-activity or H2S-production. However, Patel

et al. showed that H2S is endogenously produced in the placenta,

production rate was increased under low-oxygen levels [17].

Another study confirmed placental catalytic CBS-activity by con-

verting homocysteine [18]. So far, in PE no CBS- and CSE-activity or

H2S-production is reported. Therefore, the exact role of endoge-

nous H2S in PE needs to be elucidated.

Differential CBS mRNA expression may be gestation related, as

has been previously documented for other genes, however no

differences in CBS mRNA expression measured in first-trimester

term human placenta were reported [19]. For late-onset PE, placental CBS mRNA expression is not altered compared to healthy

pregnancies (Fig. 1A). This is in line with the growing evidence that there are differences between the pathophysiology of early- and

late-onset PE [20]. In our total study group, 3 patients with a

pregnancy complicated by IUGR were present. The data of these

patients with respect to mRNA and protein expression fitted well

within their study groups.

Fig. 1. Placental expression of CBS and CSE mRNA and protein in healthy pregnancy and pregnancy complicated by early- and late-onset preeclampsia. A. CBS mRNA expression is down regulated in the early-onset preeclampsia group, compared to the mode for delivery matched group (delivery by Cesarean section). No differences were observed in CBS mRNA expression in the late-onset PE group or in CSE mRNA expression in all four groups. There was as well no difference in mRNA between the late- and early-onset PE groups of both enzymes. B. Quantitative western blot analysis showed a significant down regulation of both CBS and CSE in all spontaneous delivery placentae, compared to the Cesarean groups. No differences were observed in expression of both enzymes between both PE groups and mode of delivery matched controls. Bands 1–4 show representative western blots for CBS in placentae derived from respectively Cesarean controls, early-

onset PE, spontaneous controls and late-onset PE. Bands 5–8 show representative western blots for CSE in the same groups. For all figures, median an interquartile range is
given. For statistical analysis, Mann–Whitney U test was used. Quantification of protein

expression was performed by measuring band intensity using ImageJ software.
CBS and CSE protein, but not mRNA expression was significantly down regulated in control and PE placentae after spontaneous delivery compared to Cesarean delivery. This is in line with a recent report of down regulation of the enzymes and reduced H2S-production in the myometrium during labor [21]. Increased turnover of the enzymes could be involved in transition of the labor process.

The fetal endothelial expression of CBS and CSE is in accord with the expressions in other organs [22]. Moreover, we showed expression of CBS by Hofbauer cells. Hofbauer cells or fetal tissue macrophages are placental immune cells; several studies suggest that Hofbauer cells play a direct role in placental vasculogenesis [23]. Although CSE has been shown to be expressed by macrophages [24], the expression of CBS by macrophages has not been reported. The expression of CBS by Hofbauer cells may be in line with the role of these cells in vasculogenesis.

In conclusion, the present study provides novel insights into the expression of H2S-producing enzymes during normal and PE. Future studies will compare the placental CBS/CSE-activity and H2S-production in pregnancies and explore the possible therapeutic role of H2S during PE.

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